Control and Management of Insect Populations by Chemosterilants

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Chemosterilants, i.e., chemical compounds that interfere with the reproduction potential of sexually reproducing organisms, can be used in three new approaches to insect control. In the sterile-insect release technique, the principal problem is to develop compounds and methods for their application that would not result in introducing harmful residues into the environment. Because of the unusual and often unique circumstances connected with releasing large numbers of sterilized insects, the residue problem and its cost-benefit aspects must be examined individually for each intended control or eradication program. In the direct application technique, chemosterilants must meet the same efficiency and safety standards required from insecticides. Combined insecticidal and sterilizing activity is characteristic for some compounds now being investigated. In the genetic technique, chemosterilants may be used for inducing heritable changes in the insect's genome under laboratory conditions, and such procedures would not present any residue problems. Only the first two chemosterilant techniques are approaching practical application, and their safety aspects require detailed evaluation and assessment.

All pests that compete with man for food and fiber and most of those that are parasites or carriers of diseases share a common characteristic: rapid reproduction. Indeed, many insects achieved the pest status primarily because their reproductive capacity was too high and their exploding populations exerted undesirable pressure on man's environment. In theory, any process that interferes with a pest's reproduction represents a potentially useful control method, but only a few such processes are sufficiently flexible and selective to become practical and competitive with established control procedures. The successful eradication of the screwworm, *Cochliomyia hominivorax* (Coquerel), from certain islands in the West Indies and from the southeastern United States (*1*) was a convincing example of a novel approach to pest control in which interference with reproduction played a key role.

Subsequent research sparked by these outstanding accomplishments and by the concern for environmental protection identified three major

areas in which the sterility concept could be utilized: the sterile-male technique, in which artificially reared male insects would be sterilized by radiation or chemicals and released into infested areas; the direct sterilization technique, in which chemosterilants would be applied like insecticides to sterilize rather than to kill pest insects; and the genetic technique, in which special mutant strains would be released to suppress the natural population. The terminology of these techniques is imprecise and subject to personal preferences, but their common feature is interference with reproduction leading to reduction or extinction of the insect population. Chemosterilants, i.e., chemical compounds that reduce or eliminate the reproductive capacity of the organism to which they are applied (*2*), may be utilized in each of the three areas mentioned, though only in the direct sterilization technique are they essential and irreplaceable. In the other two techniques, sterilization by ionizing radiation is the most common alternative. In judging the present potential and the prospective uses of chemosterilants, their role in each specific technique must be clearly understood.

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Chemosterilants in the Sterile-Male Technique

The sterile-male technique is based on a simple but eminently logical assumption that fertile females confronted with large numbers of predominantly sterile mating partners will not reproduce. Although operational and economical factors (3) impose considerable limitations on the apparent universality of the technique, ample laboratory and field experience is now available to substantiate its practicality and effectiveness (4). Clearly, the crucial step in the sterile-male technique is the production of large numbers of sexually sterile insects that would be, from the standpoint of the naturally occurring fertile female, indistinguishable from the natural males. Three factors concerning the released insects are important in this regard: changes in normal biological characteristics resulting from mass rearing, physiological and behavioral consequences of the sterilization treatment, and the effects of the release. Only the second factor will be discussed here in some detail.

Of the many possible procedures that induce sexual sterility, only radiation and chemical treatment are of practical importance. Advantages and disadvantages can be listed for either procedure (5), but in the present discussion the assumption is made that chemosterilization is the method of choice. In theory, any stage in the developing organism is subject to chemically induced physiolgical and genetic changes that result in the inability of adults to reproduce. However, experience indicates that chemosterilization becomes more successful with the insect's advancing development, and in practice only the late developmental stages and the adults are considered suitable for treatment.

Since specificity of chemosterilants is extremely variable, each insect species constitutes a new research subject in which the ultimate objective of the investigation plays a decisive role. In all practically oriented projects, a minimal sterilizing dose of the compound must be applied as uniformly as possible to a large number of insects by a safe and economically acceptable procedure.

The chemosterilant itself must meet several stringent requirements, particularly the range of doses actually administered must not kill the insect or change its mating behavior. Furthermore, if the compound is toxic or otherwise hazardous to the environment, the released sterile insects must be either free of its residue or contain only tolerable amounts of it.

Within these limits acceptable chemosterilization procedures have already been developed for

the Mexican fruit fly, *Anastrepha ludens* (Loew) (6), the house fly, *Musca domestica* L. (7), the boll weevil, *Anthonomus grandis* Boheman (8), the screwworm (9), and several species of mosquitoes (10). Basic data are available for several other pest species for which the sterile-male control technique could be developed in relatively short time.

Currently, the sterile-male technique is being used, usually as a segment of an integrated control program, against the screwworm and the pink bollworm, *Pectinophora gossypiella* (Saunders), in the United States, against the Mexican fruit fly in a locality along the U.S.-Mexican border, and against the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), in Europe and Africa. Since in all instances the insects are sterilized by radiation, it may be well to ask why chemosterilants are not being used. One of the answers is that except the bollworm, the insects used are flies (Diptera) that are relatively easily sterilized by γ-rays from a ^{60}Co source. The treatment is applied to a convenient developmental stage (pupa) of the insect, and the irradiation procedure produces no potential residue problems. Another much less obvious part of the answer is related to the somewhat unusual economic considerations connected with atomic energy installations. The often substantial costs of the radiation facility may be covered from sources not directly related to the pest control program, and a possible cost-benefit argument favoring chemical treatment may become irrelevant. Similar considerations apply also to the extent and funding of the initial research on chemosterilization and radiation sterilization. It may be expected, however, that future application of the sterile-male technique, particularly to insects that cannot be sterilized by radiation, will result in the introduction of programs employing chemosterilants. Obviously any such program will have to come to terms with the safety aspects of these compounds, and a more detailed discussion of this problem will be presented below.

Chemosterilants Applied in the Field

The possibility of treating a natural pest population with a material that would sterilize but not kill a certain percentage of the adults was suggested already in the first exposition of the sterility pest control technique (11). Subsequently, a research program directed toward discovering such chemosterilants was initiated in the U.S. Department of Agriculture, and since then well over

10,000 compounds were synthesized or procured and screened for antifertility properties in various laboratories here and abroad.

In the early 60's the enthusiasm of many commercially oriented research laboratories for chemosterilants was based mainly on the proposition that these compounds may be direct alternatives to insecticides and that they may present a permanent solution to the prevailing problem of resistance. Later disenchantment of the same circles with chemosterilants was equally precipitous. The biochemical and toxicological characteristics of then known chemosterilants made them clearly unacceptable as commercial pesticides and any other potential utilization of these compounds, particularly in the release techniques, indicated only a limited need for their production in larger quantities. Simply stated, a chemosterilant that could be directly applied to the pest-infested area in the same manner as an insecticide would have to meet precisely the same standards and requirements imposed on insecticides by nature, law, and economy.

An important point that has not been sufficiently recognized in the past is the fundamental difference between using chemosterilants in the release technique and in the field application technique. Economic considerations, particularly the cost of released sterile insects, make the release technique progressively more economical with the decreasing density of the pest insects in the infested area. To suppress an exploded population of insects effectively requires releasing a ten- to hundredfold excess of sterile insects at possibly prohibitive cost. On the other hand, broadcasting chemosterilants or insecticides is most effective when the infestation is high and when a substantial portion of the chemical is utilized for contacting the insects. Also, a chemosterilant applied directly in the field may have marked insecticidal and other physiological properties that could not be tolerated for a sterilant used in the release technique.

Though these differences suggest that the types of chemosterilants intended for use in the two mentioned techniques may be entirely different, it is nevertheless conceivable that the same compound may adequately serve both purposes. The greatest difficulty in the search for chemosterilants that could be applied directly was encountered in attempts for discovering compounds effective in males; this problem is still one of the principal challenges of chemosterilant research.

Female-sterilizing compounds, on the other hand, progressed well beyond the point of speculation, and their future practical application directly in the field is a distinct possibility. Two types of compounds, developed independently and without direct ties to chemosterilant research, belong to the latter category: juvenile hormones and their analogs or mimics, and specific growth regulators. The first group of compounds is now being developed commercially, however, not on the basis of their sterilizing properties but on the basis of their insecticidal properties which they exhibit in immature stages of certain insects. These materials were already discussed in detail in this symposium (12) and because their current use is not primarily concerned with sterilization they will be only briefly mentioned here. The second group is at present represented by only two compounds, both derivatives of urea, that are specific inhibitors of chitin synthesis. With regard to sterilization, these compounds seem to have no significant physiological effect on the adult insect's organism, but in gravid females of certain species they affect the fertilized eggs and disrupt embryogenesis after the eggs are laid. However, like the juvenile hormones, the chitin formation inhibitors are toxic to immature stages of many insects, and their future commercial development will probably emphasize this aspect of their biological activity.

Chemosterilants in the Genetic Technique

The use of mutant strains of insects for control purposes was first suggested by Serebrovsky (13) and later rediscovered independently by Curtis (14). The basic idea is very similar to that contained in Knipling's sterile-male release method (11), except that the released insects are not sterilized by chemicals or radiation but are mutants capable of introducing various detrimental genes into the existing population. Invariably, the mutant strain is derived from a single insect that has been treated by a mutagenic agent, in most instances by low doses of ionizing radiation. Chemical treatment for the same purpose, i.e., the application of substerilizing doses of mutagenic chemosterilants or of other mutagens, has not been investigated in detail, but it seems plausible that chemicals may offer a highly specific and almost infinitely flexible tool for genetic manipulation. Of course, any potential use of chemosterilants in this field would be in small-scale laboratory operations and without any direct impact on the pest control operation. Nevertheless, understanding the mechanism of genetic action of mutagenic chemosterilants is a most important prerequisite

for utilizing fully their properties in sterilization programs and for preventing their possible misuse.

Safety Aspects of Chemosterilants

The wide diversity of chemosterilants makes any generalization about their safety or hazards dubious and often misleading. Suggestions for safe handling of compounds used most frequently in laboratory and field experiments (15) may be useful when research projects with chemosterilants are being planned and initiated, but they cannot cover the steadily increasing number of candidate materials and the variety of application techniques and conditions.

Because the biochemical mechanisms that may ultimately lead to sterility and reproductive dysfunction are. extremely variable, chemosterilants can be only very inadequately classified by their structural and chemical characteristics. Table 1, an updated version of a previously published classification (2), shows the principal classes of

Table 1. Classification of insect chemosterilants.

Class	Representative compound	
	Name[a]	Reference
Alkylating agents		
Aziridines	Triethylenephosphoramide (tepa)	(16)
Alkanesulfonates	Busulfan	(17)
Nitrogen mustards	Mechlorethamine (nitrogen mustard)	(18)
Antimetabolites		
Folic acid analogs	Aminopterin	(2)
Pyrimidines	5-Fluorouracil	(2)
Nonalkylating dimethylamino compounds		
Phosphorus amides	Hexametapol (hempa)	(16)
s-Triazines	Hexamethylmelamine (hemel)	(19)
Dithiazoles	3,5-Bis(dimethylamino)-1,2,4-dithiazolium chloride	(20)
Tin compounds	Triphenyltin hydroxide	(21)
Boron compounds	Benzeneboronic acid	(22)
Miscellaneous agents		
Alkaloids	Captothecin	(23)
Antibiotics	Anthramycin	(24)
Juvenoids	Methyl 3,7,11-trimethyl-7,11-dichloro-2-dodeconoate	(25)
Growth inhibitors	1-(4-Chlorophenyl)-3-(2,6-difluorobenzoyl)urea (TH 6040)	(26)

[a]Wherever applicable, *Merck Index* (8th ed.) headings were used as first names; common names are parenthesized.

chemosterilants. It is impossible to predict which of these classes will assume practical importance in the future, but if completion of successful field experiments is taken as an indicator, the alkylating agents become the most prominent group. As was discussed in a review of this class of chemosterilants (17), the toxicological properties of alkylating agents make their direct application in the field undesirable. However, in the release technique and possibly in the genetic techniques, these compounds are clearly outstanding because of their high effectiveness in the male organism and their apparently unlimited spectrum of activity in insects and other animals. Since high biological activity and lack of specificity are danger signals from the toxicological and environmental standpoint, any application of alkylating agents must be carefully scrutinized for two possible hazards: the first lies in handling the compound during the chemosterilization step; the second potential hazard is the residues of chemosterilants contained in the sterilized and released insects. The mass rearing and sterilization procedures must be under direct control of the operators, and thus safe procedures for handling even very toxic materials can always be designed. The residue problem, however, is much more difficult to resolve, particularly because it frequently touches on the general problem of toxicological threshold.

As an example we may consider the recently completed Pilot Boll Weevil Eradication Experiment (27). The chemosterilant used was 1,4-butanediol dimethanesulfonate (busulfan), an alkylating agent well known and widely used as cancer chemotherapeutic agent. Gas chromatographic analysis of the treated weevils showed no residues of busulfan (8), but since the minimum amount of the compound detectable by the method used was ca. 150 ng/weevil, it cannot be excluded that the released weevils did contain some busulfan. But let us assume that there indeed is a residue and that each released weevil contains 100 ng of busulfan. At an application of 100 sterile weevils/acre, the highest possible amount of busulfan reaching the cotton field would be 10 μg/acre or about 6 mg per square mile. Would this contamination constitute a hazard? There may be no absolute answer to this question but perhaps a more realistic problem lies in the possibility of any concentration of these minute contaminants. With due regard to the highly questionable validity of any statement concerning a class of compounds as broad as the alkylating agents, the chances for accumulation of any of these reactive compounds in the environment are negligible. Nevertheless, it is

necessary to carefully consider the specific circumstances of any program utilizing this type of compounds and to search for any potential problems related to this possibility.

Two other classes of chemosterilants shown in Table 1 are of importance. The nonalkylating phosphorus amides (16), particularly their best-known representative hempa (hexamethylphosphoric triamide), are a potentially useful group of chemosterilants. Toxicological data on hempa (28) show only moderate mammalian toxicity, but the compound, or perhaps its metabolite (29), is mutagenic, and the considerations regarding its use in any of the sterility pest control techniques will not be qualitatively different from those described earlier for alkylating agents.

The most recent example of the broad category of sterilants, included in Table 1, is 1-(5-chlorophenyl)-3-(2,6-difluorobenzoyl)urea (TH 6040), a compound that inhibits the synthesis of chitin in developing insects (30). Since the mechanism of action of this material is apparently entirely different from that of the mutagenic sterilants, its potential use could include direct broadcasting with all the problems characteristic to applications of insecticides. However, the toxicity of TH 6040 to immature stages is much more pronounced than its sterilizing activity, and the compound is apt to be remembered as an insecticide rather than a chemosterilant. A similar though not as well defined potential exists for certain insect hormones and their derivatives. Again, their principal activity is disruption of the insect's normal development, and their possible environmental impact has been discussed elsewhere (12). Nevertheless, it is important to consider these compounds as chemosterilants to gain a full perspective of the role sterility and its induction may play in new approaches to pest control.

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